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Effects of fruit extract of sponge plant (*Luffa cylindrica*) on proximate composition of African Catfish (*Clarias gariepinus*) juveniles

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ABSTRACT
The effects of extracts from the fruits of Sponge plant (*Luffa cylindrica*) on the proximate composition of juveniles of *Clarias gariepienus* was investigated under laboratory conditions using static renewal bioassay for 56 days. The fish with average length and weight of 13.12±1.01cm and 14.72±3.63g respectively were exposed to sub-lethal concentrations of 883mg/L, 1766mg/L, 3532mg/L and 7063mg/L of extracts from the fruit of *L. cylindrica*. The physicochemical parameters of water contaminated with sub-lethal concentrations of *Luffa cylindrica* were examined. The pH of the test media was slightly acidic, which tend to increase as concentration of *Luffa cylindrica* extract increases. Dissolved Oxygen decreased while Conductivity and Total Dissolved Solids increased with increase in concentrations *of Luffa cylindica* extract. The changes in the physicochemical parameters were significant differences across the different concentrations (P<0.05). There were significant reductions (P<0.05) in protein, lipid, fiber and ash content (14.21±0.48% to 9.78±0.48%; 2.38±0.02% to 1.83±0.10%; 4.60±0.12% to 1.51±0.11% and 3.25±0.08% to 1.71±0.01 respectively) as the concentration of the extract increases. However, moisture content and carbohydrate content (74.67±0.61% to 82.55±0.43% and 1.44±0.48% to 2.62±0.48% respectively) significantly increased (P<0.05) from the control to the treated groups. The study revealed that the extract of *Luffa cylindrical* have adverse effects on the nutritional composition of *Clarias gariepinus*. Therefore, consumption of fish killed with piscicides should be discouraged because of the reduced nutritional values.
Keywords: Piscicides, *Luffa cylindrica,* Proximate composition, sponge plant

INTRODUCTION

In the quest to catch more fish to meet protein demand of the nation, fisher folks have resorted to the use of obnoxious fishing methods of which the use of ichthyotoxic plants is chief among them. Plant parts have been shown to cause death of fish and changes in biochemical responses of *Channa punctatus* [1]; haematological and histopathological effects on *Clarias gariepinus* [2, 3]; and nutritional value (proximate composition) of these fish may also be affected.

Many piscicides are used in Nigeria by local fishermen, however little have been documented on their toxicity, especially on the quality of the fish killed by these piscicides. Paucity of information exits on the effects of piscicides on histology, haematology and other physiological processes in fish; most especially on the proximate composition of fish. A widely used piscicide is Luffa (*Luffa cylindrica* or *Luffa aegyptiaca*). It is commonly called vegetable sponge, sponge gourd, loofa, sponge plant, bath sponge or dish cloth gourd, and is a member of cucurbitaceous family [4].

Luffa cylindrica is a plant that is widely distributed within the savannah belts of West Africa including Nigeria where it is reported to have domestic and medicinal values [5, 6]. Local fishers in Nigeria use the fruits of this plant to harvest fish from water bodies for human consumption. In a study by Ekpendu *et al*., [7] to evaluate the botanical piscicides available in six geopolitical zones of Nigeria, they found out that *Luffa cylindrica* was the most commonly used piscicide in three of the zones, Rivers (South South), Lagos (South West) and Adamawa State (North East) respectively. However, there is no available information on the safety of humans consuming fish caught using the plant.

In the study [8] to investigate the chronic toxicity of fenthion (pesticide) on proximate composition of *Cyprinus carpio*; it was evident that *C. carpio* exposed to 0.38, 0.193 and 0.096 mg/L Fenthion for 60 days showed significant reduction in glycogen and protein contents of muscle and liver tissues; decrease in liver somatic index (LSI) values with increase in lipid and moisture content. A decrease in crude protein, fat and carbohydrate contents of *Etroplus maculatus* body was observed, when exposed to various sublethal concentrations of monocrotophos; whereas the ash levels increased with increasing concentrations [9].

Although the piscicidal potentials of *Luffa cylindrica* are generally known among local fishermen, there are little documented reports on the toxicity of *Luffa cylindica* on African catfish. There is no reported work done on the effects of this piscicides on the proximate composition of fish. Therefore, there is need to study the effects of this piscicide (*Luffa cylindrical* fruit) on the proximate composition of *Clarias gariepinus* juvenile.

MATERIALS AND METHODS

Collection and Preparation of Experimental Plant and Fish

Luffa cylindrica fruits were collected in swampy area around the University of Port Harcourt field and were identified using appropriate keys [10]. The fresh fruits of *L. cylindrica* were sliced into smaller sizes using a knife, pulverized with a mortar and pestle without adding water and strained with 30µm mesh-size sieve. The sub-lethal concentrations of *L. cylindrica* fruit extract to use were realized after series of pilot studies and acute toxicity tests. Five concentrations, 0.00 mg/L (Control), 883 mg/L, 1766 mg/L, 3532 mg/L and 7063 mg/L) of *L. cylindrica* fruit extract were used for the exposure.

Clarias gariepinus juveniles were obtained from University of Port Harcourt Faculty of Agriculture Demonstration Farm, Port Harcourt, Nigeria. The mean weight and length were 14.72±3.63g and 13.12±1.01cm respectively. The juveniles were transported to the laboratory in the early hours of the day when the atmospheric temperature was still relatively stable. In the laboratory, they were immediately transferred into holding tanks and acclimatized for 14 days before the commencement of the experiment. The holding tanks were cleaned and water renewed once in three days [11]. The test fish were fed twice daily based on 5% of their body weight during the period of acclimatization, using formulated fish feed (40% protein) from African Regional Aquaculture Centre (ARAC), Aluu, Port Harcourt.

Fifteen plastic aquaria were used for this study (five treatments with 3 replicates each), and with ten juveniles of C*. gariepinus* arbitrarily exposed to different concentrations of *L. cylindrica* fruit extract for 56 days. During this period, freshly prepared test solution was added to maintain the concentration level after the waste was removed. Fish were fed twice daily based on 5% of their body weight with ARAC feed. Physicochemical parameters such as temperature, pH, dissolved oxygen (DO), Conductivity and Total dissolved solid were monitored using standard procedures as described by APHA, [12] on weekly basis.

Determination of Proximate Components of Fish

The Association of Official Analytical Chemists (AOAC) method was used to determine the ash, moisture, lipid, and crude protein contents (Kjeldahl Method) [13]. While the total carbohydrate content was estimated by subtracting the sum of the weight of moisture, ash, protein and lipid from the total dry matter and this was reported as Nitrogen-free extractives (NFE by differences) [14]. All determinations were done in triplicates and the mean recorded.

Data Analysis

Statistically data was analyzed using one way ANOVA and Duncan's multiple range tests to compare the mean values of the samples. Results were tested for statistically significant differences at 0.05 levels. All statistics were done with the aid of IBM SPSS version 20.0. Results were presented as mean ± standard deviation.

RESULTS

Physicochemical Parameters

The physicochemical parameters of water contaminated with sub-lethal concentration of *Luffa cylindrica* fruit extract is presented in Table 1. The pH of the test media was slightly acidic, ranging from 6.63±0.38 for the control to 5.95±0.40 for the highest concentration. It showed that acidity tend to increase as concentration of *Luffa cylindrica* extract increases. Although the differences in pH values across the different concentrations were not statistically significant ($F=3.27$, $p>0.05$); the values obtained for the different concentrations were slightly below WHO/FMEVN standard.

The temperature ranges from 27.07±0.31^{0}C to 27.48±0.62^{0}C and the differences in the temperature was also not statistically significant ($F= 0.32$, $P>0.05$).

Dissolved oxygen tend to decreasing with increase in concentration of fruit extract in 0.00mg/L (6.1±00.20 mg/L), 883 mg/L (5.10±0.28 mg/L), 1766 mg/L (4.98±0.28 mg/L), 3532 mg/L (4.38±0.56 mg/L) and 7063 mg/L (4.18±0.50 mg/L). The differences in the DO values were statistically significant ($F=11.05$, $p<0.05$) but were within WHO/FMEVN standard.

The TDS and Conductivity showed an increasing trend, the TDS values obtained for the different concentrations of *Luffa cylindrica* fruit extract indicated that there were significant differences across the different concentrations ($F=33.37$, $p<0.05$). The control recorded the least value of 9.97±1.85 mg/L while the highest concentration had a value of 34.23±5.53 mg/L, these values were within the WHO/FMEVN limit of 250 mg/L. The result for the conductivity showed that the values obtained across the treatments were within WHO/FMEVN standard. The differences in conductivity values were statistically significant ($F=14.54$, $p<0.05$).

Moisture Content

Proximate Composition

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The proximate composition of *Clarias gariepinus* exposed to different concentrations of *Luffa cylindrica* is presented in Table 2. Moisture content of *Clarias gariepinus* exposed to the different sublethal concentration of fruit extract showed that 7063 mg/L had the highest moisture content (82.55±0.43%) followed by 3532 mg/L (80.76±0.95%). The 1766 mg/L, 883 mg/L and control had 78.82±0.50%, 76.43±0.60% and 74.67±0.81% respectively. The differences in moisture content were statistically significant ($P < 0.05$).

Crude Protein Content

The result of the crude protein content of the five concentrations of *Luffa cylindirca* fruit extract showed that there was significant difference at $p < 0.05$. The control had the highest protein content (14.21±0.61%). The 883 mg/L, 1766 mg/L, 3532 mg/L and 7063 mg/L had crude protein content as follows; 13.42±0.22%, 11.83±0.30%, 10.74±0.24% and 9.78±0.24% respectively (Table 2).

Ash Content

The ash content of *Clarias gariepinus* exposed to fruit extract of *Luffa cylindrica* shows significant difference ($p<0.05$) between the concentrations. The fish in control (3.25±0.08%) recorded the highest ash content, followed by 883 mg/L (3.02±0.17%) but the difference between the control and 883 mg/L was not statistically significant ($P>0.05$). The ash

DISCUSSION

The Physicochemical parameters of the water were affected by the fruit extract, although not significant, but led to stress factors that were reflected in behavioural distress like restlessness, increased respiratory rate, gulping of air and loss of balance of the test fish.

Lipid, protein and carbohydrate, which constitute the major components of the body, play an important role in energy breakdown. This is affected by factors like toxins and toxicants [9]. Decrease in lipid, protein and fiber content of *Clarias gariepinus* juvenile exposed to different sublethal concentrations of toxicants are linked through a common metabolic pathway *i.e.*, the tricarboxylic acid (TCA) cycle, [15].

Significant differences in the ash (carcass) of *Clarias gariepinus* exposed to *L. cylindrica* extract may be the direct result of reduced food consumption and food conversion efficiency as was observed in rohu and *Etroplus maculatus* juveniles exposed to sublethal concentrations of monocrotophos [8, 9]. Similar effects were observed in *Etroplus maculatus* exposed

content of 1766 mg/L, 3532 mg/L and 7063 mg/L were 2.58±0.36%, 2.29±0.36% and 1.71±0.01% respectively (Table 2).

Lipid Content

Table 2 shows the lipid content of *Clarias gariepinus* exposed to different concentrations of *Luffa cylindrica* fruit extract. The values obtained showed that the control had the highest lipid content (2.38±0.02%) while concentration 7063 mg/L recorded the least value of 1.83±0.10%. The differences in lipid values were statistically significant ($P < 0.05$).

Carbohydrate Content

The highest concentration (7063 mg/L) recorded the highest carbohydrate value (2.62±0.48) followed by 3532 mg/L, 1766 mg/L and 883 mg/L with 2.36±0.48%, 2.15±0.48 and 1.53±0.48% respectively while the control recorded the least value of 1.44±0.48%. The results of carbohydrate for *Clarias gariepinus* juveniles exposed to *Luffa cylindrica* extract exhibited significant differences across the treatments ($p<0.05$) (Table 2).

Fiber Content

There were significant differences ($p < 0.05$) in the values obtained for the fiber content across the different treatments (Table 2). The fish in control recorded the highest fiber content with 4.60±0.12% and those in 7063 mg/L concentration recorded the least value with 1.51±0.11%.
to phosphamidon and *Anabas testudineus* exposed to monocrotophos and phosphamidon[9, 16].

In the present study, there was a significant decrease ($p<0.05$) in protein content which correlated with concentrations of the fruit extract. Protein is the source of energy during chronic conditions of stress [9] and a number of studies have reported a decline in protein composition in different organs of fishes treated with toxicants [8,16,17,18]. Depletion in protein level in the body might be due to diversion of energy when the animal was under toxic condition as reported by Ugwu *et al.* [16].

Lipid was found to decrease considerably in this work which may be due to utilization of stored and circulatory cholesterol and other lipid fractions in the treated fish to counteract toxic effects produced. This result conforms closely with Dahunsi *et al*. [19] who observed a decrease level of fat in *Clarias gariepinus* exposed to sublethal concentrations of chemical additive. Rani *et al*. [20] and Shankar and Kulkarni [21], also observed the same trend in *Notopterus notopterus* during stress.

Carbohydrate is the principal and immediate source of energy. From the result, it was observed that chronic exposure to *L. cylindrica* significantly increased the carbohydrate content of *Clarias gariepinus*. This result disagrees with the works of Muralidharan [7] when he exposed *Cyprinus carpio* to fenthion; and Sulekha and Mercy [22] when they exposed *Etroplus maculatus* to sublethal concentrations of monocrotophos. The increase in carbohydrate components may be as a result of the activities of the cortisol that regulates and mobilizes energy. Cortisol mobilizes energy by attaching into the body's stored fat (in the form of triglycerides which is unsaturated fat) and moving it from one location to another, or delivering it to hungry tissues such as working muscle. When an organism is faced with stressful conditions, cortisol provides the body with protein for energy production through gluconeogenesis, [23]. Additionally, cortisol can move fat from storage depots and relocate it to fat cell deposits deep in the abdomen [24]. Cortisol also aids adipocytes (baby fat cells) to grow up into mature fat cells [25].

The percentage of water in the body of any living organism is a good indicator of its relative content of energy, proteins and lipids. The lower the percentage of water in a fish, the greater the lipid, protein and energy density of the fish [26, 27, 28]. This pattern was observed in this study. The increase in the moisture content of the exposed fish were statistically significant and this is in agreement with the work of Muralidharan [8] who also noticed an increasing trend in moisture content of *Cyprinus carpio* exposed to sublethal concentrations of fenthion for 60 days. Other authors have also reported similar trend: Aberoumand and Pourshafi, [21] reported 68.6% to 77.1%, Ravichandran *et al* [29] reported values of 77.93 to 82.70%, and Fawole *et al* [28] reported 55.95 to 68.80% which is lower than what was obtained in this study. Hence in the present study, the cause for rise in moisture content could be due to the subsequent utilization of tissue proteins.

CONCLUSIONS

The study has shown that *Luffa cylindrica* fruit extract have adverse effects on the nutritional composition of *Clarias gariepinus*. The use of fruit extract of *L. cylindrica* will not only lead to contamination of an ecological system, thereby posing great threat to fish and other non-target organisms, but will lead to nutritional deficiency when fishes killed by it are being consumed. Therefore, consumption of fish killed with piscicides should be discouraged as they contain reduced nutrients.

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Table 1: Physico-Chemical Parameters of sublethal concentrations of *L. cylindrica* fruit extract

Parameters	Control	Treatment1 883mg/L	Treatment2 1766 mg/L	Treatment3 3532 mg/L	Treatment4 7063 mg/L	WHO/FMEVN Standard
pH	6.63±0.38a	6.35±0.21ab	6.08±0.17a	5.98±0.17b	5.95±0.40b	6.5 – 8.5
DO (mg/L)	6.10±0.20a	5.10±0.28ab	4.98±0.28b	4.38±0.56b	4.18±0.50b	> 4.0
Temperature (^{0}C)	27.07±0.31a	27.45±0.78a	27.40±0.56a	27.48±0.62a	27.48±0.62a	20 – 30
TDS (mg/L)	9.97±1.05d	12.45±1.34d	17.55±1.57cd	21.98±1.84bc	34.23±5.53a	250
Conductivity (uS/cm)	22.83±7.52c	21.50±7.78c	35.15±3.71b	33.13±2.89b	48.63±5.16a	100

Note: Values in each row with the same superscript are not significantly different at P >0.05; FMENV - Federal Ministry of Environment in Nigeria

Table 2: Proximate composition of *Clarias gariepinus* fingerlings exposed to sublethal concentrations of fruit extract of *L. cylindrica*.

Parameters	Control 0.00mg/L	Treatment1 883mg/L	Treatment2 1766mg/L	Treatment3 3532mg/L	Treatment4 7063mg/L
Carbohydrate	$1.44{\pm}0.48^{e}$	$1.53{\pm}0.48^{d}$	$2.15{\pm}0.48^{c}$	$2.36{\pm}0.48^{b}$	2.62 ± 0.48^{a}
Protein	$14.21{\pm}0.61^{a}$	$13.42{\pm}0.22^{b}$	$11.83{\pm}0.30^{c}$	$10.74{\pm}0.24^{d}$	$9.78{\pm} 0.24^{e}$
Moisture	74.6 ± 0.81^{e}	$76.43{\pm}0.60^{d}$	$78.82{\pm}0.50^{c}$	80.76 ± 0.95^{b}	$82.55{\pm}0.43^{a}$
Ash	3.25 ± 0.08^{a}	3.02 ± 0.17^{a}	2.58 ± 0.36^{b}	$2.29{\pm}0.36^{b}$	$1.71{\pm} 0.01^{c}$
Lipid	2.38 ± 0.02^{a}	2.17 ± 0.10^{b}	$2.10{\pm}0.11^{b}$	1.90 ± 0.10^{c}	$1.83{\pm}0.10^{c}$
Fiber	4.60 ± 0.12^{a}	2.84 ± 0.47^{b}	$2.56{\pm} 0.32^{b}$	$2.01{\pm} 0.05^{c}$	1.51 ± 0.11^{d}

Note: Values in each row with the same superscript are not significantly different at P >0.05